

BioMycoX® Mycoplasma Elimination Kit

(Light/ Medium/ Heavy contamination)

Cat. No. E-01/ E-02/ E-03
Storage Temperature 4-8°C

The *BioMycoX*® Mycoplasma Elimination kit has been developed to quickly and completely eliminate mycoplasma contamination from cell cultures. The potential effects of mycoplasma contamination in cell culture are major concern in research, diagnostics, and production of biological products. Mycoplasma contamination decreases the quantity of product produced and its quality and results in inconsistency and lack of reproducibility of the experiment. Current methods for the inactivation or elimination of mycoplasma in cell cultures are antibiotic-based. Antibiotic therapies in particular do not always result in a successful and permanent elimination of the contaminants. Moreover antibiotics exhibit cytotoxic properties in that they are capable of modifying the metabolism of eukaryotic cells and promote the development of resistant mycoplasma strains. The *BioMycoX*® Mycoplasma Elimination Reagent is a combination of biological agents that reliably and completely eliminate mycoplasma contamination. Compared with other elimination products, the *BioMycoX*® Mycoplasma Elimination kit is more successful and dose not cause any changes in normal cell characteristics. The *BioMycoX*® Mycoplasma Elimination Kit is suitable for the elimination of *Mollicutes* and related organisms (*Mycoplasma*, *Acholeplasma*, *Ureaplasma*, *Spiroplasma*, and *Entomoplasma*) in cell and virus cultures.

1. Kit Components

Cat. No	E-01	E-02	E-03
	Light contamination	Medium contamination	Heavy contamination
BioMycoX Reagent I (green cap)	200 µl	200 µl X 2	200 µl X 3
BioMycoX Reagent II (blue cap)	200 µl	200 µl X 2	200 µl X 3
Cell strainer	2 ea	4 ea	6 ea

2. Mycoplasma Elimination Protocols

	Treatment No.
Light contamination	2 times
Medium contamination	4 times
Heavy contamination	6 times
Use 100 µl of reagent I and II for each treatment	

- 1) Prepare enough amounts of mycoplasma infected cells.
- 2) Trypsinize mycoplasma infected cells (80 ~ 90% confluent) through a usual method. Check for complete detachment of cells.
- 3) Centrifuge the cell suspension at 1,000rpm for 5 minutes. Decant the supernatant.
- 4) Resuspend cell pellet in 10ml of the growth medium containing 5% FBS.
- 5) Make the cell suspension to a cell density of 5X10⁵~1X10⁶cells/ml.

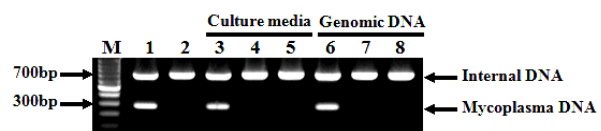
- 6) Strain out the cell suspensions with a **cell strainer** included in the kit. If cell suspension does not pass the filter, gently pipette up and down.
- 7) Transfer 10ml of cell suspensions into new 15ml conical tube.
- 8) Add 100ul of *BioMycoX*® Reagent I and 100ul of *BioMycoX*® Reagent II. Mix it carefully.
- 9) Transfer it to 100mm culture dish or T75 culture flask and incubate the flask as normal for 3 days.

*Depending on the extent of mycoplasma contamination and the types of cells, cytotoxicity may be detected during cell cultivation. And the cytotoxicity can be observed in cells ranging from 0 to 70%. However, cells turn to normal after the complete elimination of mycoplasma.

- 10) Follow steps 11~18 in case of reach to 80~90% confluence. Follow steps 19~20 in case of slow growth of cell.
- 11) Trypsinize cells at a usual method. Check for complete detachment of cells.
- 12) Centrifuge the cell suspension at 1,000rpm for 5 minutes. Decant the supernatant.
- 13) Resuspend cell pellet in 10ml of the growth medium containing 5% FBS.
- 14) Make the cell suspension to a cell density of 5X10⁵~1X10⁶cells/ml.
- 15) Strain out the cell suspensions with a **cell strainer** included in the kit. If cell suspension does not pass the filter, gently pipette up and down
- 16) Transfer 10ml of cell suspensions into new 15ml conical tube
- 17) Add 100ul of *BioMycoX*® Reagent I and 100ul of *BioMycoX*® Reagent II. Mix it carefully.
- 18) Transfer it to 100mm culture dish or T75 culture flask and incubate the flask as normal for 2~3 days (80%~90% confluent).
- 19) Remove old medium and add new medium containing 5% FBS into culture dish.
- 20) Add 100ul of *BioMycoX*® Reagent I and 100ul of *BioMycoX*® Reagent II into the culture dish. And incubate the dish as normal for 2~3 days.
- 21) Confirm the success of the treatment by testing with *BioMycoX*® Mycoplasma PCR Detection Kit.

***Caution :** For medium or heavy contamination, treat the elimination reagents continuously.

3. Result



- M: 100bp DNA ladder
 1: Positive control (mycoplasma control DNA)
 2: Negative control (DW)
 3, 6: Mycoplasma infected cell / before treatment
 4, 7: Mycoplasma infected cell / 1 passage after treatment
 5, 8: Mycoplasma infected cell / 10 passages after treatment